METHOD FOR THE TREATMENT OF MULTIPLE SCLEROSIS BY INHIBITING IL-17 ACTIVITY

The present invention relates generally to methods of treating multiple sclerosis and more specifically to the use of inhibitors of IL-17 activity for the manufacture of a
5   medicament for the treatment of multiple sclerosis.

Interleukin 17 (IL-17), also known as CTLA-8 or IL-17A, is a pro-inflammatory cytokine which stimulates the secretion of a wide range of other cytokines from various non-immune cells. IL-17 is capable of inducing the secretion of IL-6, IL-8, PGE2, MCP-1 and G-CSF by adherent cells like fibroblasts, keratinocytes, epithelial and endothelial cells and is
10   also able to induce ICAM-1 surface expression, proliferation of T cells, and growth and differentiation of CD34+ human progenitors into neutrophils when cocultured in presence of irradiated fibroblasts (Fossiez et al., 1998, Int.Rev.Immunol. 16, 541-551). IL-17 is predominantly produced by activated memory T cells and acts by binding to a ubiquitously distributed cell surface receptor (IL-17R) (Yao et al.,1997, Cytokine, 9, 794-800). A
15   number of homologues of IL-17 have been identified which have both similar and distinct roles in regulating inflammatory responses. For a review of IL-17 cytokine/receptor families see Dumont, 2003, Expert Opin. Ther. Patents, 13, 287-303.

        IL-17 may contribute to a number of diseases mediated by abnormal immune responses, such as rheumatoid arthritis and air-way inflammation, as well as organ transplant
20   rejection and antitumour immunity. Inhibitors of IL-17 activity are well known in the art, for example an IL-17R:Fc fusion protein was used to demonstrate the role of IL-17 in collagen-induced arthritis (Lubberts et al., J.Immunol. 2001,167, 1004-1013) and neutralising polyclonal antibodies have been used to reduce peritoneal adhesion formation (Chung et al., 2002, J.Exp.Med., 195, 1471-1478). Neutralising monoclonal antibodies are
25   commercially available (R&D Systems UK).

        Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS), which is believed to result from a coordinated autoimmune attack against myelin antigens. There is considerable clinical and pathological heterogeneity in MS patients and the sequence of events that initiate the disease remain largely unknown.
30   The clinical progression of MS may be largely attributed to three disease processes; inflammation, demyelination and axonal loss / neurodegeneration.

        Immune mediated inflammatory lesions within the CNS are thought to result primarily from an infiltration of autoreactive CD4+ lymphocytes (Th1) which recognise

myelin proteins presented on MHC class II molecules by antigen presenting cells. This
interaction causes stimulation of Th1 cells which release proinflammatory cytokines (mainly
TNF-α & IFN-γ) resulting in proliferation of T-cells, activation of B-cells and macrophages,
upregulation of adhesion molecules and disruption of the blood-brain barrier. Such events

5   ultimately lead to loss of oligodendrocytes & axons and the formation of a demyelinated
plaque. This is the hallmark of MS and consists of a demarcated lesion where myelin sheaths
are completely lost and demyelinated axons are embedded in glial scar tissue. Demyelination
may also occur as a consequence of specific recognition and opsonization of myelin antigens
by autoantibodies. The most important target antigen is suggested to be myelin

10   oligodendrocyte protein (MOG), which is present on the surface of the myelin sheath.
Destruction of antibody-opsonized myelin is then accomplished either by complement or
activated macrophages. Axonal loss and neurodegeneration subsequent to inflammation are
thought to be responsible for the accumulation of irreversible neurological impairment,
characteristic of secondary progressive MS.

15   The clinical features of MS vary from headaches and blurred vision to severe ataxia,
blindness and paralysis. MS affects all ages but first symptoms generally occur between 18
and 50 years and disease duration has been estimated at >25 years with a significant
proportion of patients dying from causes unrelated to MS. In the majority of patients (~80%)
the disease takes a relapsing-remitting (RR-MS) course with exacerbation of symptoms,

20   which is rapid in onset (hours to days) followed by a slower recovery. The frequency and
duration of relapses are unpredictable but average 1.5 per year and can be followed by
complete recovery. With time, recovery from relapses may not be complete and a gradual
worsening of disease occurs. This worsening of disease is independent of relapse rate and is
classified as secondary progressive MS (SP-MS), accounting for approximately 10% of MS

25   patients. The remaining 10% of MS patients have a primary progressive (PP-MS) course
where disability worsens at a steady rate from onset of the disease.

           Currently licensed therapies are the beta-interferons; Interferon beta-1b (*Betaseron*;
Berlex), Interferon beta-1a (*Avonex*; Biogen, *Rebif*; Serono) and glatimer acetate (*Copaxone*;
Teva). These agents have been shown to reduce relapse rate during the relapsing-remitting

30   phase of the disease in approximately 30% of patients. There is currently no method
available for identifying the responder population before therapy. Intravenous steroids
(prednisolone is most commonly used) are used to hasten remission after relapse but do not
have long term efficacy. The anti-cancer agent, mitoxantrone (*Novantrone*), is approved as

an immunosuppressant in progressive-relapsing and secondary-progressive patients, but its use and dose is limited by cardiotoxicity. In Europe azathioprine has also been used as an immunosuppressant.

Prescribing decisions seem to be driven by evidence-based medicine and a recent report by the American Association of Neurologists (Goodin DS et al; Neurology 2002 Jan 22;58(2):169-78) is a key document. The consensus amongst many neurologists is that early, aggressive therapy with beta-interferons was desirable in increasing the time to first relapse and limiting the overall disease load, although it was recognised that there was no evidence that this approach showed long-term benefit on EDSS score (a measure of disease-related disability). Beta-interferons were seen as sub-optimal therapy and glatirimer acetate as having a different mechanism of action, which may allow it to be used (alone or in combination) in patients that do not respond to interferons. Individualised therapy based on mechanistic (MRI, genetic, neurological) markers of disease was seen as a worthwhile goal, as were therapies with a novel mechanism of action. There is currently no satisfactory diagnostic marker for multiple sclerosis.

There is a clear need for disease modifying therapies. Agents with different mechanisms of action are needed and may allow therapy to be tailored to different stages of the disease. An orally active agent is yet to be licensed in the relapsing-remitting form of the disease and this would represent a clear improvement over current therapy if significant efficacy was associated with the mechanism. Furthermore, there is a clear requirement for therapies that show efficacy in the primary or secondary progressive phases of the disease and have a reasonable side-effect profile.

Whether IL-17 plays any kind of role in the pathogenesis of MS is unknown. Microarray analysis of MS lesions obtained at autopsy have revealed increased transcripts of many different genes encoding inflammatory cytokines, including, IL-17 (Lock *et al.*, 2002, Nature Medicine, 8, 500-508). An increased number of IL-17 expressing mononuclear cells have been detected in blood and cerebrospinal fluid from patients with MS (Matusevicius *et al.*, 1999, Multiple Sclerosis, 5, 101-104) but as the authors point out cytokine mRNA expression is not necessarily identical to cytokine protein production.

Surprisingly we have been able to demonstrate that inhibitors of IL-17 activity are active in an animal model of MS. Specifically we have been able to demonstrate that an anti-IL-17 antibody that inhibits IL-17 activity is active in animal models of MS. Hence, the present invention provides a method for the treatment and/or prophylaxis of MS comprising

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administering a therapeutically effective amount of an inhibitor of IL-17 activity. The invention also provides the use of an inhibitor of IL-17 activity for the manufacture of a medicament for the treatment and/or prophylaxis of multiple sclerosis.

5   The term 'IL-17 activity' as used herein refers to the spectrum of activity understood in the art for IL-17 for example, the induction of secretion of IL-6 or IL-8 from fibroblasts by IL-17 (Yao *et al.*, 1995, Journal of Immunology, 155,5483-5486).

An inhibitor of IL-17 activity according to the present invention is an agent that interferes with the activity of IL-17, in particular the activity of IL-17 in MS. Particularly preferred are agents which interfere with the activity of IL-17 in MS in humans. Inhibitors

10   according to the present invention may partially or completely inhibit IL-17 activity. Inhibitors of use in the present invention include without limitation, inhibitors that are capable of interacting with (*e.g.* binding to, or recognising) IL-17 or the IL-17 receptor (IL-17 R) or a nucleic acid molecule encoding IL-17 or IL-17R, or are capable of inhibiting the expression of IL-17 or IL-17 R or are capable of inhibiting the interaction between IL-17 and

15   IL-17R. Such inhibitors may be, without limitation, antibodies, nucleic acids (*e.g.* DNA, RNA, antisense RNA and siRNA), carbohydrates, lipids, proteins, polypeptides, peptides, peptidomimetics, small molecules and other drugs.

Examples of suitable inhibitors include, but are not limited to, a synthetic functional fragment of the IL-17 receptor that binds to IL-17 and interferes with binding to the native

20   IL-17 receptor, an antibody that binds to IL-17 or to the IL-17 receptor and interferes with IL-17 receptor-ligand interaction, an antisense nucleic acid molecule that specifically hybridizes to mRNA encoding IL-17 or the IL-17 receptor or a small molecule or other drug which inhibits the activity of IL-17 or its receptor.

Inhibitors of IL-17 activity are well known in the art as are methods of identifying

25   and producing such inhibitors. Examples include, IL-17R:Fc fusion proteins (Lubberts *et al.*, J.Immunol. 2001,167, 1004-1013) and neutralising antibodies (Chung *et al.*, 2002, J.Exp.Med., 195, 1471-1478; Ferretti, 2003, Journal of Immunology, 170, 2106-2112). Agents that may be suitable inhibitors can be selected from a wide variety of candidate agents. Examples of candidate agents include but are not limited to, nucleic acids (*e.g.* DNA

30   and RNA), carbohydrates, lipids, proteins, polypeptides, peptides, peptidomimetics, small molecules and other drugs. Agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods

requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is suited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, Anticancer Drug Des.

5   12:145; U.S. 5,738,996; and U.S. 5,807,683).

Examples of suitable methods based on the present description for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:6909; Erb *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann *et al.*, 1994, J. Med. Chem. 37:2678; Cho *et al.*, 1993, Science 261:1303;

10   Carrell *et al.*, 1994, Angew. Chem. Int. Ed. Engl. 33:2059; Carell *et al.*, 1994, Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop *et al.*, 1994, J. Med. Chem. 37:1233.

Libraries of compounds may be presented, for example, in solution (*e.g.* Houghten, 1992, Bio/Techniques 13:412-421), or on beads (Lam, 1991, Nature 354:82-84), chips (Fodor, 1993, Nature 364:555-556), bacteria (US 5,223,409), spores (US 5,571,698;

15   5,403,484; and 5,223,409), plasmids (Cull *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310).

In one example, the inhibitor for use in the present invention may be a nucleic acid. In

20   particular IL-17 or IL-17R nucleic acid molecules may be used as anti-sense molecules, to alter the expression of their respective polypeptides by binding to complementary nucleic acids. IL-17 or IL-17R nucleic acids may be obtained using standard cloning techniques from for example genomic DNA or cDNA or can be synthesised using well known and commercially available techniques. The IL-17 or IL-17R nucleic acids may contain one or more

25   nucleotide substitutions, additions or deletions into the nucleotide sequence of an IL-17 or IL-17R nucleic acid. Standard techniques known to those of skill in the art can be used to introduce mutations, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis. An antisense nucleic acid according to the present invention includes a IL-17 or IL-17R nucleic acid capable of hybridising by virtue of some sequence complementarity

30   to a portion of an RNA (preferably mRNA) encoding the respective polypeptide. The antisense nucleic acid can be complementary to a coding and/or non-coding region of an mRNA encoding such a polypeptide. Most preferably, the antisense nucleic acids result in inhibition of the expression of the IL-17 or IL-17R polypeptide. Thus, the present invention

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provides a method for the treatment and/or prophylaxis of MS comprising administering a
therapeutically effective amount of an inhibitor of IL-17 activity wherein the inhibitor
comprises at least eight nucleotides that are antisense to a gene or cDNA encoding a IL-17 or
IL-17R polypeptide. The invention also provides the use of nucleic acids comprising at least

5   eight nucleotides that are antisense to a gene or cDNA encoding a IL-17 or IL-17R
polypeptide for the manufacture of a medicament for the treatment and/or prophylaxis of
MS.

       Most preferably, an inhibitor for use in the treatment and/or prophylaxis of MS is an
antibody that interacts with (i.e. binds to or recognises) IL-17 or its receptor and inhibits the

10   activity of IL-17. Accordingly, there is provided the use of an antibody that inhibits the
activity of IL-17 for the manufacture of a medicament for the treatment and/or prophylaxis
of MS. Also provided is a method of treatment and/or prophylaxis of MS in a subject
comprising administering to said subject a therapeutically effective amount of an antibody
that inhibits the activity of IL-17.

15   In one example the antibodies selectively interact with IL-17. Selectively interacting
with (e.g. recognising or binding to) means that the antibodies have a greater affinity for IL-
17 polypeptides than for other polypeptides. Examples of suitable antibodies are those that
inhibit the activity of IL-17 by binding to IL-17 in such a manner as to prevent it being
biologically active, for example by preventing the binding of IL-17 to its receptor.

20   Accordingly, there is provided by the present invention the use of an anti-IL-17 antibody for
the manufacture of a medicament for the treatment and/or prophylaxis of MS. Also provided
is a method of treatment and/or prophylaxis of MS in a subject comprising administering to
said subject a therapeutically effective amount of an anti-IL-17 antibody.

       In another example the antibodies selectively interact with the IL-17 receptor.

25   Selectively interacting with (e.g. recognising or binding to) means that the antibodies have a
greater affinity for the IL-17 receptor polypeptide than for other polypeptides. Examples of
suitable antibodies are those that inhibit the activity of IL-17 by preventing IL-17 mediated
signalling from the receptor, for example by preventing IL-17 from binding to the IL-17
receptor. Accordingly, there is provided by the present invention the use of an anti-IL-17R

30   antibody for the manufacture of a medicament for the treatment and/or prophylaxis of MS.
Also provided is a method of treatment and/or prophylaxis of MS in a subject comprising
administering to said subject a therapeutically effective amount of an anti-IL-17R antibody.

IL-17 or IL-17 receptor polypeptides or cells expressing said polypeptides can be used to produce antibodies which specifically recognise said polypeptides. The IL-17 and IL-17 R polypeptides may be 'mature' polypeptides or biologically active fragments or derivatives thereof. IL-17 and IL-17 R polypeptides may be prepared by processes well

5   known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. IL-17 or IL-17R polypeptides may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag. Antibodies

10   generated against these polypeptides may be obtained by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D.M.Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986. Many warm-blooded animals, such as rabbits, mice, rats, sheep, chickens, cows or pigs may be immunised. However, mice, rabbits, pigs

15   and rats are generally preferred.

Anti-IL-17 and anti-IL-17 receptor antibodies for use in the present invention include · whole antibodies and functionally active fragments or derivatives thereof and may be, but are not limited to, polyclonal, monoclonal, multi-valent, multi-specific, humanized or chimeric antibodies, single chain antibodies, Fab fragments, Fab' and F(ab')$_2$ fragments, fragments

20   produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Antibodies include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.* molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (*e.g.* IgG, IgE, IgM, IgD and IgA) or subclass of

25   immunoglobulin molecule.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy,

30   pp77-96, Alan R Liss, Inc., 1985).

Antibodies for use in the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by for

example the methods described by Babcook, J. *et al.*, 1996, Proc. Natl. Acad. Sci. USA
93(15):7843-7848 and in WO92/02551.

Humanized antibodies are antibody molecules from non-human species having one or
more complementarity determining regions (CDRs) from the non-human species and a
5   framework region from a human immunoglobulin molecule (see, *e.g.* US 5,585,089).

Chimeric antibodies are those antibodies encoded by immunoglobulin genes that
have been genetically engineered so that the light and heavy chain genes are composed of
immunoglobulin gene segments belonging to different species. These chimeric antibodies
are likely to be less antigenic. Bivalent antibodies may be made by methods known in the
10   art (Milstein *et al.*, 1983, Nature 305:537-539; WO 93/08829, Traunecker *et al.*, 1991,
EMBO J. 10:3655-3659). Multi-valent antibodies may comprise multiple specificities or
may be monospecific (see for example WO 92/22853).

The antibodies for use in the present invention can also be generated using various
phage display methods known in the art and include those disclosed by Brinkman *et al.* (in J.
15   Immunol. Methods, 1995, 182: 41-50), Ames *et al.* (J. Immunol. Methods, 1995, 184:177-
186), Kettleborough *et al.* (Eur. J. Immunol. 1994, 24:952-958), Persic *et al.* (Gene, 1997
187 9-18), Burton *et al.* (Advances in Immunology, 1994, 57:191-280) and WO 90/02809;
WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401;
and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047;
20   5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.
Techniques for the production of single chain antibodies, such as those described in US
4,946,778 can also be adapted to produce single chain antibodies to IL-17 or IL-17R
polypeptides. Also, transgenic mice, or other organisms, including other mammals, may be
used to express humanized antibodies.

25   Antibody fragments and methods of producing them are well known in the art, see
for example Verma *et al.*, 1998. Journal of Immunological Methods, 216, 165-181.

Particular examples of antibody fragments for use in the present invention are Fab'
fragments which possess a native or a modified hinge region. A number of modified hinge
regions have already been described, for example, in US 5,677,425, WO9915549, and
30   WO9825971 and these are incorporated herein by reference

Further examples of particular antibody fragments for use in the present invention
include those described in International patent applications PCT/GB2004/002810,

PCT/GB2004/002870 and PCT/GB2004/002871 (all filed on 1st July 2004). In particular the modified antibody Fab fragments described in International patent application PCT/GB2004/002810 are preferred. These Fab fragments comprise a heavy and light chain pair, V_H/C_H1 and V_L/C_L covalently linked through interchain cysteines in the heavy and light

5   chain constant regions and are characterised in that the heavy chain constant region terminates at the interchain cysteine of C_H1. The term 'interchain cysteine' refers to a cysteine in the heavy or light chain constant region that would be disulphide linked to a cysteine in the corresponding heavy or light chain constant region encoded in a naturally occurring germline antibody gene.   In particular the interchain cysteines are a cysteine in

10   the constant region of the light chain (C_L) and a cysteine in the first constant region of the heavy chain (C_H1) that are disulphide linked to each other in naturally occurring antibodies. Examples of such cysteines may typically be found at position 214 of the light chain and position 233 of the heavy chain of human IgG1, position 127 of the heavy chain of human IgM, IgE, IgG2, IgG3, IgG4 and position 128 of the heavy chain of human IgD and IgA2B,

15   as defined by Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA. In murine IgG, interchain cysteines may be found at position 214 of the light chain and position 235 of the heavy chain. It will be appreciated that the exact positions of these cysteines may vary from that of naturally occurring antibodies if any modifications, such as deletions, insertions and/or substitutions have been made to the antibody Fab fragment. These antibody Fab fragments may be

20   prepared by any suitable method known in the art.   For example, the antibody Fab fragment may be obtained from any whole antibody, especially a whole monoclonal antibody, using any suitable enzymatic cleavage and/or digestion techniques, for example by treatment with pepsin or papain and c-terminal proteases. Preferably these antibody Fab fragments are

25   prepared by the use of recombinant DNA techniques involving the manipulation and re-expression of DNA encoding antibody variable and constant regions. Standard molecular biology techniques may be used to modify, add or delete further amino acids or domains as desired.   Any alterations to the variable or constant regions are still encompassed by the terms 'variable' and 'constant' regions as used herein. Preferably PCR is used to introduce a

30   stop codon immediately following the codon encoding the interchain cysteine of C_H1, such that translation of the C_H1 domain stops at the interchain cysteine. Methods for designing suitable PCR primers are well known in the art and the sequences of antibody C_H1 domains are readily available (Kabat et al., supra). Alternatively stop codons may be introduced

using site-directed mutagenesis techniques such as those described in White (Ed.), PCR

Protocols: Current Methods and Applications (1993). In one example the constant regions in

these fragments are derived from IgG1 and the interchain cysteine of C_L is at position 214 of

the light chain and the interchain cysteine of C_H1 is at position 233 of the heavy chain.

5   Examples of human and murine constant region sequences for use in these fragments are

provided in SEQ ID Nos 1-4 and Figure 13; human heavy chain constant region C_H1 which

terminates at the interchain cysteine (SEQ ID NO:1); human light chain constant region

(SEQ ID NO:2); murine heavy chain constant region C_H1 which terminates at the interchain

cysteine (SEQ ID NO:3); murine light chain constant region (SEQ ID NO:4).

10   If desired an antibody for use in the present invention may be conjugated to one or

more effector molecule(s). The term effector molecule as used herein includes, for

example, antineoplastic agents, drugs, toxins, biologically active proteins, for example

enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers,

nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides,

15   particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups

such as fluorescent compounds or compounds which may be detected by NMR or ESR

spectroscopy. In one example, anti-IL-17 or anti IL-17 R antibodies can be conjugated to an

effector molecule, such as a cytotoxic agent, a radionuclide or drug moiety to modify a given

biological response. For example, the therapeutic agent may be a drug moiety which may be

20   a protein or polypeptide possessing a desired biological activity. Such moieties may include,

for example and without limitation, a toxin such as abrin, ricin A, pseudomonas exotoxin, or

diphtheria toxin, a protein such as tumour necrosis factor, α-interferon, β-interferon, nerve

growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic

agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response

25   modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-

6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony

stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

         In another example the effector molecules may be cytotoxins or cytotoxic agents

including any agent that is detrimental to (*e.g.* kills) cells. Examples include taxol,

30   cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide,

vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione,

mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids,

procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include radionuclides such as ^{111}In and ^{90}Y, Lu^{177}, Bismuth^{213}, Californium^{252}, Iridium^{192} and Tungsten^{188}/Rhenium^{188}; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53; Thorpe *et al.*, 1982 , Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). In one example, the antibody or fragment thereof is fused via a covalent bond (*e.g.* a peptide bond), at optionally the N-terminus or the C-terminus, to an amino acid sequence of another protein (or portion thereof; preferably at least a 10, 20 or 50 amino acid portion of the protein). Preferably the antibody, or fragment thereof, is linked to the other protein at the N-terminus of the constant domain of the antibody. Recombinant DNA procedures may be used to create such fusions, for example as described in WO 86/01533 and EP 0392745.

In another example the effector molecule may increase half-life *in vivo*, and/or decrease immunogenicity and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules include polymers and proteins such as albumin and albumin binding proteins. Examples of suitable polymers include anv synthetic or naturally occurring substantially water-soluble, substantially non-antigenic polymer including, for example, optionally substituted straight or branched chain polyaikyiene, polyalkenylene, or polyoxyalkylene polymers or branched or unbranched polysaccharides, e.g. a homo- or hetero- polysaccharide such as lactose, amylose, dextran or glycogen. Particular optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups. Particular examples of synthetic polymers include optionally substituted straight or branched chain

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poly(ethyleneglycol), poly(propyleneglycol), poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol). Preferably the polymer is a polyalkylene oxide such as polyethylene glycol (PEG).

In one example antibodies for use in the present invention are attached to

5   poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant

10   DNA methods. See for example US 5,219,996. Multiple sites can be used to attach two or more PEG molecules. Preferably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used.

15   Preferably, the antibody is a modified Fab fragment, such as a Fab' which is PEGylated, i.e. has PEG (poly(ethyleneglycol)) covalently attached thereto, e.g. according to the method disclosed in EP 0948544 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris

20   and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. The total amount of PEG attached to the fragment may be varied as desired, but will generally be in an average molecular weight range from 250 to 100,000Da,

25   preferably from 5,000 to 50,000Da, more preferably from 10,000 to 40,000Da and still more preferably from 20,000 to 40,000Da The size of PEG may in particular be selected on the basis of the intended use of the product, for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545).

30   In one embodiment PEG is attached to a cysteine in the hinge region of a Fab'. In one example, a PEG modified Fab' fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to each of the amine groups on the lysine residue may be attached a

methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000
Da. The total molecular weight of the PEG attached to the Fab' fragment may therefore be
approximately 40,000 Da.

5   In another preferred embodiment an antibody fragment for use in the present
invention is a PEGylated (i.e. has PEG (poly(ethyleneglycol)) covalently attached thereto)
Fab fragment as described in International Application Number PCT/GB2004/002810 (filed
on 1st July 2004). This PEGylated Fab fragment is a Fab fragment in which the heavy chain
terminates at the interchain cysteine of C_H1 and the PEG attached to the fragment, preferably
PEG-maleimide, is covalently linked to the interchain cysteine of C_L and the interchain
10   cysteine of C_H1. Preferably the interchain cysteine of C_L is at position 214 of the light chain
and the interchain cysteine of C_H1 is at position 233 of the heavy chain. As discussed above
the total amount of PEG attached to the fragment may be varied as desired. In one example
each polymer attached to the Fab preferably has a molecular weight of approximately 20,000
Da. For example, the molecular weight may be 15,000-25,000Da, or preferably 18,000-
15   22,000Da, and even more preferably 19,000-21,000Da. The total molecular weight of the
PEG attached to the antibody is therefore approximately 40,000 Da.

         PEG is attached to these fragments by first reducing the interchain disulphide bond
between the interchain cysteines of C_L and C_H1 and subsequently attaching the PEG to the
free thiols. Once PEG is attached to the interchain cysteines there is no interchain disulphide
20   linkage between the heavy and light chain. Suitable reducing agents for reducing the
interchain disulphide bond are widely known in the art for example those described in Singh
et al., 1995, Methods in Enzymology, 251, 167-73. Particular examples include thiol
based reducing agents such as reduced glutathione (GSH), β-mercaptoethanol (β-ME), β-
mercaptoethylamine (β-MA) and dithiothreitol (DTT). Other methods include using
25   electrolytic methods, such as the method described in Leach *et al.*, 1965, Div. Protein.
Chem, 4, 23-27 and using photoreduction methods, such as the method described in Ellison
et al., 2000, Biotechniques, 28 (2), 324-326. Preferably however, the reducing agent is a
non-thiol based reducing agent, preferably one of the trialkylphosphine reducing agents
(Ruegg UT and Rudinger, J., 1977, Methods in Enzymology, 47, 111-126; Burns J *et al.*,
30   1991, J.Org.Chem, 56, 2648-2650; Getz *et al.*, 1999, Analytical Biochemistry, 273, 73-80;
Han and Han, 1994, Analytical Biochemistry, 220, 5-10; Seitz *et al.*, 1999, Euro.J.Nuclear
Medicine, 26, 1265-1273), particular examples of which include tris(2-
carboxyethyl)phosphine (TCEP), tris butyl phosphine (TBP), tris-(2-cyanoethyl) phosphine,

tris-(3-hydroxypropyl) phosphine (THP) and tris-(2-hydroxyethyl) phosphine. Most preferred are the reducing agents TCEP and THP. It will be clear to a person skilled in the art that the concentration of reducing agent can be determined empirically, for example, by varying the concentration of reducing agent and measuring the number of free thiols

5   produced. Typically the reducing agent is used in excess over the antibody fragment for example between 2 and 1000 fold molar excess. Preferably the reducing agent is in 2, 3, 4, 5, 10, 100 or 1000 fold excess. In one embodiment the reductant is used at between 2 and 5mM.

The reduction and PEGylation reactions may generally be performed in a solvent, for

10   example an aqueous buffer solution such as acetate or phosphate, at around neutral pH, for example around pH 4.5 to around pH 8.5, typically pH 4.5 to 8, suitably pH6 to 7. The reactions may generally be performed at any suitable temperature, for example between about 5°C and about 70°C, for example at room temperature. The solvent may optionally contain a chelating agent such as EDTA, EGTA, CDTA or DTPA. Preferably the solvent

15   contains EDTA at between 1 and 5mM, preferably 2mM. Alternatively or in addition the solvent may be a chelating buffer such as citric acid, oxalic acid, folic acid, bicine, tricine, tris or ADA. The PEG will generally be employed in excess concentration relative to the concentration of the antibody fragment. Typically the PEG is in between 2 and 100 fold molar excess, preferably 5, 10 or 50 fold excess.

20   Where necessary, the desired product containing the desired number of PEG molecules may be separated from any starting materials or other product generated during the production process by conventional means, for example by chromatography techniques such as ion exchange, size exclusion, protein A, G or L affinity chromatography or hydrophobic interaction chromatography.

25   To identify inhibitors of IL-17 activity a number of different approaches may be taken by those skilled in the art. In one example inhibitors are identified by first identifying agents that interact with IL-17 or IL-17R and subsequently testing those agents to identify those that inhibit IL-17 activity. In one such example the agent is an antibody.

Agents that interact with IL-17 or IL-17R may be identified using any suitable

30   method, for example by using a cell-free or cell-based assay system where the IL-17 or IL-17R polypeptide is contacted with a candidate agent and the ability of the candidate agent to interact with the polypeptide is determined. Preferably, the ability of a candidate agent to interact with a IL-17 or IL-17R polypeptide is compared to a reference range or control. If

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desired, this assay may be used to screen a plurality (*e.g.* a library) of candidate agents using a plurality of IL-17 or IL-17R polypeptide samples. In one example of a cell free assay, a first and second sample comprising native or recombinant IL-17 or IL-17R polypeptide are contacted with a candidate agent or a control agent and the ability of the candidate agent to

5   interact with the polypeptide is determined by comparing the difference in interaction between the candidate agent and control agent. Preferably, the polypeptide is first immobilized, by, for example, contacting the polypeptide with an immobilized antibody which specifically recognizes and binds it, or by contacting a purified preparation of polypeptide with a surface designed to bind proteins. The polypeptide may be partially or

10   completely purified (*e.g.* partially or completely free of other polypeptides) or part of a cell lysate. Further, the polypeptide may be a fusion protein comprising the IL-17 or IL-17R polypeptide or a biologically active portion thereof and a domain such as glutathionine-S-transferase or the Fc region of IgG1. Alternatively, the polypeptide can be biotinylated using techniques well known to those of skill in the art (*e.g.* biotinylation kit, Pierce Chemicals;

15   Rockford, IL). The ability of the candidate agent to interact with the polypeptide can be determined by methods known to those of skill in the art for example, ELISA, BIAcore™, Flow cytometry or fluorescent microvolume assay technology (FMAT). In another example where a cell-based assay is used, a population of cells expressing IL-17 or IL-17 R is contacted with a candidate agent and the ability of the candidate agent to interact with the

20   polypeptide is determined. Preferably, the ability of a candidate agent to interact with IL-17 or IL-17 R is compared to a reference range or control. The cell, for example, can be of eukaryotic origin (*e.g.* yeast or mammalian) and can express the IL-17 or IL-17 R polypeptide endogenously or be genetically engineered to express the polypeptide. In some instances, the IL-17 or IL-17R polypeptide or the candidate agent is labelled, for example

25   with a radioactive label (such as ^{32}P, ^{35}S or ^{125}I) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between a polypeptide and a candidate agent. Alternative methods such as ELISA, flow cytometry and FMAT may also be used.

       Agents which inhibit IL-17 activity may be identified by any suitable method, for

30   example by:

       (i)   comparing the activity of IL-17 in the presence of a candidate agent with the activity of said polypeptide in the absence of the candidate agent or in the presence of a control agent; and

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(ii)   determining whether the candidate agent inhibits activity of IL-17.

Such assays can be used to screen candidate agents, in clinical monitoring or in drug development.

As described above, agents may be pre-screened where appropriate to identify agents
5   (e.g. an antibody) that interact with IL-17 or IL-17R prior to screening those agents which bind for their ability to inhibit IL-17 activity.

In one example a cell-based assay system is used to identify agents capable of inhibiting the activity of IL-17. In one particular example the assay used to identify inhibitors of IL-17 activity is the standard IL-6 release assay from fibroblasts (Yao *et al.,*
10   1995, Journal of Immunology, 155,5483-5486). Potential inhibitors are added to the assay and IL-6 release determined by ELISA. Inhibition is therefore measured as a reduction in IL-6 release compared to controls.

In another example inhibitors of IL-17 may down-regulate the expression of the IL-17 or IL-17R polypeptide, for example antisense inhibitors. Such inhibitors may be
15   identified by any method known in the art. In one example such inhibitors are identified in a cell-based assay system. Accordingly, a population of cells expressing a IL-17 or IL-17R polypeptide or nucleic acid are contacted with a candidate agent and the ability of the candidate agent to alter expression of the IL-17 or IL-17R polypeptide or nucleic acid is determined by comparison to a reference range or control. In one example, populations of
20   cells expressing a IL-17 or IL-17R polypeptide are contacted with a candidate agent or a control agent and the ability of the candidate agent to alter the expression of the IL-17 or IL-17R polypeptides or nucleic acids is determined by comparing the difference in the level of expression of the IL-17 or IL-17R polypeptides or nucleic acids between the treated and control populations of cells. If desired, this assay may be used to screen a plurality (e.g. a
25   library) of candidate agents. The cell, for example, can be of eukaryotic origin (*e.g.* yeast or mammalian) and can express an IL-17 or IL-17R polypeptide endogenously or be genetically engineered to express a IL-17 or IL-17R polypeptide. The ability of the candidate agents to alter the expression of a said polypeptides or nucleic acids can be determined by methods known to those of skill in the art, for example and without limitation, by flow cytometry,
30   radiolabelling, a scintillation assay, immunoprecipitation, Western blot analysis, Northern blot analysis or RT-PCR.

Agents that inhibit the activity of IL-17 may be identified or further tested, for example to determine therapeutically effective amounts in one or more animal models. Examples of

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suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represents a model of MS.

In one example where the agent inhibits the expression of IL-17 or IL-17R, a first and second group of mammals are administered with a candidate agent or a control agent and the

5   ability of the candidate agent to inhibit the expression of IL-17 or IL-17R polypeptide or nucleic acid is determined by comparing the difference in the level of expression between the first and second group of mammals. Where desired, the expression levels of the IL-17 or IL-17R polypeptides or nucleic acid in the first and second groups of mammals can be compared to the level of IL-17 or IL-17R polypeptide or nucleic acid in a control group of

10   mammals. The candidate agent or a control agent can be administered by means known in the art (*e.g.* orally, rectally or parenterally such as intraperitoneally or intravenously). Changes in the expression of a polypeptide or nucleic acid can be assessed by the methods outlined above.

In another example, the inhibition of IL-17 activity can be determined by monitoring

15   an amelioration or improvement in disease symptoms, a delayed onset or slow progression of the disease, for example but without limitation, a reduction in paralysis. Techniques known to physicians familiar with MS can be used to determine whether a candidate agent has altered one or more symptoms associated with the disease.

A number of different models of MS are known in the art ('t Hart and Amor 2003,

20   Current Opinion in Neurology 16:375-83). In particular, experimental autoimmune encephalomyelitis (EAE) in ABH mice is considered to be a relevant model for MS in humans (Baker et al., 1990. Journal of Neuroimmunology, 28:261-270). Both acute and relapsing-remitting models have been developed.

As discussed herein, inhibitors of IL-17 activity can be used in the treatment and/or

25   prophylaxis of MS. For such use the agents will generally be administered in the form of a pharmaceutical composition.

Also provided is a pharmaceutical composition comprising an inhibitor of IL-17 activity and a pharmaceutically acceptable carrier.

The term 'treatment' includes either therapeutic or prophylactic therapy. When a

30   reference is made herein to a method of treating or preventing a disease or condition using a particular inhibitor or combination of inhibitors, it is to be understood that such a reference is intended to include the use of that inhibitor or combination of inhibitors for the manufacture of a medicament for the treatment and/or prophylaxis of MS.

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The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. This composition may be in any suitable form (depending upon the desired method of administering it to a patient).

5   The inhibitors of use in the invention are preferably administered to a subject by a variety of other routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intrathecally and intracerebroventricularly. The most suitable route for administration in any given case will depend on the particular inhibitor, the subject, and the nature and severity of the disease and the physical condition of the subject.

10   The inhibitors of use in the invention may be administered in combination, e.g. simultaneously, sequentially or separately, with one or more other therapeutically active compounds, which may be for example other anti-MS therapies or anti-cancer therapies.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose. Such a unit

15   may contain for example but without limitation, 750mg/kg to 0.1mg/kg depending on the condition being treated, the route of administration and the age, weight and condition of the subject.

Pharmaceutically acceptable carriers for use in the invention may take a wide variety of forms depending, e.g. on the route of administration.

20   Compositions for oral administration may be liquid or solid. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Oral liquid preparations may contain suspending agents as known in the art.

25   In the case of oral solid preparations such as powders, capsules and tablets, carriers such as starches, sugars, microcrystalline cellulose granulating agents, lubricants, binders, disintegrating agents, and the like may be included. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are generally employed. In addition to the common dosage

30   forms set out above, active agents of the invention may also be administered by controlled release means and/or delivery devices. Tablets and capsules may comprise conventional carriers or excipients such as binding agents for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch,

calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated by standard aqueous or non-aqueous techniques according to methods well known in normal

5   pharmaceutical practice.

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active agent, as a powder or granules, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-

10   in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association the active agent with the carrier, which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active agent with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product

15   into the desired presentation. For example, a tablet may be prepared by compression or moulding, optionally with one or more accessory ingredients.

Pharmaceutical compositions suitable for parenteral administration may be prepared as solutions or suspensions of the active agents of the invention in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol,

20   liquid polyethylene glycols, and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include aqueous or non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes

25   which render the composition isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Extemporaneous injection solutions, dispersions and suspensions may be prepared from sterile powders, granules and tablets.

Pharmaceutical compositions can be administered with medical devices known in

30   the art. For example, in a preferred embodiment, a pharmaceutical composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in US 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention

include: US 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; US 4,486,194, which discloses a therapeutic device for administering medicaments through the skin; US 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; US 4,447,224, which

5   discloses a variable flow implantable infusion apparatus for continuous drug delivery; US 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and US 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

Pharmaceutical compositions adapted for topical administration may be formulated as

10   ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, impregnated dressings, sprays, aerosols or oils, transdermal devices, dusting powders, and the like. These compositions may be prepared via conventional methods containing the active agent. Thus, they may also comprise compatible conventional carriers and additives, such as preservatives, solvents to assist drug penetration, emollients in creams or ointments and

15   ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the composition. More usually they will form up to about 80% of the composition. As an illustration only, a cream or ointment is prepared by mixing sufficient quantities of hydrophilic material and water, containing from about 5-10% by weight of the compound, in sufficient quantities to produce a cream or ointment having the desired

20   consistency.

Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active agent may be delivered from the patch by iontophoresis.

25   For applications to external tissues, for example the mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active agent may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active agent may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

30   Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

Pharmaceutical compositions adapted for topical administration to the eye include eye

drops wherein the active agent is dissolved or suspended in a suitable carrier, especially an

aqueous solvent. They also include topical ointments or creams as above.

Pharmaceutical compositions suitable for rectal administration wherein the carrier is

5   a solid are most preferably presented as unit dose suppositories. Suitable carriers include

cocoa butter or other glyceride or materials commonly used in the art, and the suppositories

may be conveniently formed by admixture of the combination with the softened or melted

carrier(s) followed by chilling and shaping moulds. They may also be administered as

enemas.

10   The dosage to be administered of an inhibitor of IL-17 activity will vary according to

the particular inhibitor, the type of MS, the subject, and the nature and severity of the disease

and the physical condition of the subject, and the selected route of administration; the

appropriate dosage can be readily determined by a person skilled in the art. For the

treatment and/or prophylaxis of MS in humans and animals pharmaceutical compositions

15   comprising antibodies can be administered to patients (e.g., human subjects) at

therapeutically or prophylactically effective dosages (e.g. dosages which result in inhibition

of MS and/or relief of MS symptoms) using any suitable route of administration, such as

injection and other routes of administration known in the art for clinical products, such as

antibody-based clinical products.

20   The compositions may contain from 0.1% by weight, preferably from 10-60%, or

more, by weight, of the inhibitor of the invention, depending on the method of

administration.

It will be recognized by one of skill in the art that the optimal quantity and spacing of

individual dosages of an inhibitor of the invention will be determined by the nature and

25   extent of the condition being treated, the form, route and site of administration, and the age

and condition of the particular subject being treated, and that a physician will ultimately

determine appropriate dosages to be used. This dosage may be repeated as often as appropriate.

If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in

accordance with normal clinical practice.

30   In another example, where the inhibitor is a nucleic acid this may be administered via

gene therapy (see for example Hoshida, T. et al., 2002, Pancreas, 25:111-121; Ikuno, Y.

2002, Invest. Ophthalmol. Vis. Sci. 2002 43:2406-2411; Bollard, C., 2002, Blood 99:3179-

3187; Lee E., 2001, Mol. Med. 7:773-782). Gene therapy refers to administration to a

subject of an expressed or expressible nucleic acid. In one example this is either the IL-17 or the IL-17R nucleic acid or portions thereof. Any of the methods for gene therapy available in the art can be used according to the present invention.

5   Delivery of the therapeutic nucleic acid into a patient can be direct *in vivo* gene therapy (*i.e.* the patient is directly exposed to the nucleic acid or nucleic acid-containing vector) or indirect *ex vivo* gene therapy (*i.e.* cells are first transformed with the nucleic acid *in vitro* and then transplanted into the patient).

For example for *in vivo* gene therapy, an expression vector containing the IL-17 or IL-17R nucleic acid may be administered in such a manner that it becomes intracellular, *i.e.*
10   by infection using a defective or attenuated retroviral or other viral vectors as described, for example, in US 4,980,286 or by Robbins *et al.*, 1998, Pharmacol. Ther. 80:35-47.

The various retroviral vectors that are known in the art are such as those described in Miller *et al.* (1993, Meth. Enzymol. 217:581-599) which have been modified to delete those retroviral sequences which are not required for packaging of the viral genome and
15   subsequent integration into host cell DNA. Also adenoviral vectors can be used which are advantageous due to their ability to infect non-dividing cells and such high-capacity adenoviral vectors are described in Kochanek (1999, Human Gene Therapy, 10:2451-2459). Chimeric viral vectors that can be used are those described by Reynolds *et al.* (1999, Molecular Medicine Today, 1:25 –31). Hybrid vectors can also be used and are described by
20   Jacoby *et al.* (1997, Gene Therapy, 4:1282-1283).

Direct injection of naked DNA or through the use of microparticle bombardment (*e.g.* Gene Gun®; Biolistic, Dupont) or by coating it with lipids can also be used in gene therapy. Cell-surface receptors/transfecting compounds or through encapsulation in liposomes, microparticles or microcapsules or by administering the nucleic acid in linkage to a peptide
25   which is known to enter the nucleus or by administering it in linkage to a ligand predisposed to receptor-mediated endocytosis (See Wu & Wu 1987, J. Biol. Chem., 262:4429-4432) can be used to target cell types which specifically express the receptors of interest.

In *ex vivo* gene therapy, a gene is transferred into cells *in vitro* using tissue culture and the cells are delivered to the patient by various methods such as injecting subcutaneously,
30   application of the cells into a skin graft and the intravenous injection of recombinant blood cells such as haematopoietic stem or progenitor cells.

Cells into which a IL-17 or IL-17R nucleic acid can be introduced for the purposes of gene therapy include, for example, epithelial cells, endothelial cells, keratinocytes,

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fibroblasts, muscle cells, hepatocytes and blood cells. The blood cells that can be used

include, for example, T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils,

eosinophils, megakaryotcytes, granulocytes, haematopoietic cells or progenitor cells, and the

like.

5   In a one aspect, the pharmaceutical composition of the present invention comprises an

IL-17 or IL-17R nucleic acid, said nucleic acid being part of an expression vector that

expresses an IL-17 or IL-17R polypeptide or chimeric protein thereof in a suitable host. In

particular, such a nucleic acid has a promoter operably linked to the polypeptide coding

region, said promoter being inducible or constitutive (and, optionally, tissue-specific).

10   The invention will now be described with reference to the following examples, which

are merely illustrative and should not in any way be construed as limiting the scope of the

present invention.

Figures

15   **Figure 1.** Effect of Ab#13mIgG1 on clinical disease when dosed from day -1 through the

acute phase (black arrowheads, days −1, 6, 13, 20). Average clinical score (+/- sd) plotted

against day of disease induction (a) and average change in weight from day 0 (b).

Figure 2. Analysis of the acute phase of disease following dosing of Ab#13mIgG1 through

the acute phase.

20   **Figure 3.** Analysis of the relapse phase of disease following dosing of Ab#13mIgG1

through the acute phase.

Figure 4. Effect of Ab#13mIgG1 on clinical disease when dosed through the relapse phase

(days 28, 35, 42 and 49, black arrowheads). Average clinical score (+/- sd) versus day of

disease induction (a) and average change in weight from day 0 (b).

25   **Figure 5.** Analysis of the acute phase of disease following dosing of Ab#13mIgG1 through

the relapse phase.

Figure 6. Analysis of the relapse phase of disease following dosing of Ab#13mIgG1 through

the relapse phase.

Figure 7. Effect of Ab#13 Fab-di-PEG and Ab#13 mIgG1 on clinical disease when dosed

30   through the relapse phase (black arrowheads represent dosing days). Average clinical score

(+/- sd) plotted against day of disease induction (a) and average change in weight from day 0

(b).

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Figure 8. Analysis of acute phase prior to dosing with Ab#13 Fab-di-PEG and Ab#13
mIgG1.

Figure 9. Analysis of relapse phase following dosing with Ab#13 Fab-di-PEG and Ab#13
mIgG1 during remission.

5   **Figure 10.** Effect of Ab#13mIgG1 on clinical disease when dosed from day -1 (black
arrowheads represent dosing days, prophylactically (a) and therapeutically (b).

Figure 11. Analysis of prohylactic dosing regime.

Figure 12. Analysis of therapeutic dosing regime.

Figure 13. Human and murine constant regions.

10

Examples

Example 1. Isolation of an anti-IL-17 antibody

15   Rabbits were immunised three times with human IL-17 and then twice with mouse IL-17.
Using a haemolytic plaque assay with biotinylated sheep red blood cells coated with murine
IL-17 via streptavidin, 9 antibody genes were isolated using the methods described by
Babcook *et al.*, 1996, Proc.Natl.Acad.Sci, 93, 7843-7848 and in WO92/02551. The
antibody genes were expressed in CHO cells and the recombinant antibodies screened for

20   their ability to neutralise murine IL-17 in a bioassay using mouse 3T3-NIH cells (Yao *et al.*,
1995, Immunity, 3:811-821). All the antibodies in the panel neutralised murine IL-17 in this
assay and one antibody, m170013 (Ab#13) was selected for *in vivo* testing. For testing the
efficacy of the antibody in EAE, a chimeric IgG (Ab#13 mIgG1) was produced using the
rabbit variable region from antibody m170013 and mouse constant regions.

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Example 2. Effect of Ab#13mIgG1 on the symptoms of EAE

The MS model, experimental autoimmune encephalomyelitis (EAE), was used essentially as
described by Baker et al., 1990. Journal of Neuroimmunology, 28:261-270.

Female ABH mice 8-10 weeks of age (Harlan) were immunised with mouse spinal cord

30   homogenate (SCH, 3.33mg/ml) in complete freund's adjuvant by subcutaneous
immunisation in either flank (150μl/site) on days 0 and 7.

i) Dosing over the acute phase

Two groups were dosed with antibody at 10mg/kg , sc on days -1, 6, 13 and 20. One group (n=14) was dosed with Ab#13 mIgG1 the other (n=13) with 101.4 (isotype control).

5   ii) Dosing over the relapsing phase

A total of 30 mice were followed through the acute phase of disease and on day 27 analysis of the acute phase of disease was performed to select two groups with similar disease profiles in the acute phase (day of onset, peak disease score, cumulative clinical score and weight loss). Two groups of 12 mice were selected for dosing with antibody at 10mg/kg , sc

10   on days 28, 35, 42 and 49. One group was dosed with Ab#13 mIgG1) the other with 101.4 (isotype control).

Weights and clinical scores were recorded daily by an assessor blinded to treatment and terminal EDTA-Plasma collected.

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Clinical score scale

0	Normal
0.25	Tail dragging
0.5	Partial tail paralysis
1	Complete tail paralysis
2	Incomplete hind limb paralysis
3	Complete hind paralysis / incontinence
4	Front limb paralysis / loss of righting reflex

20

25   Statistics

Pairwise comparisons of clinical scores and day of onset were performed using Mann-Whitney U test, analysis of incidence was performed with Fishers exact test, analysis of maximum weight loss was performed using Students' T test.

Results

30

i) Dosing during acute phase: A significant delay in the onset of the acute phase and a reduced severity and incidence of first relapse was observed (Figures 1, 2 and 3). Figure 2 shows that Ab#13 mIgG1dosed through the acute phase had no effect on the incidence of disease in the acute phase (Ab#13 mIgG1, 13/14 with disease vs 13/13 for isotype control,

35   101.4). The only statistically significant effect was a delay in the onset of the acute phase of disease Figure 2 a, p=0.0039, Mann-Whitney U test. No effects were seen on weight loss (b), maximum clinical score (c) or cumulative clinical score (d) in the acute phase. Figure 3

shows that Ab#13 mIgG1 dosed through the acute phase caused a significant reduction in the incidence of the relapse phase of disease (Ab#13 mIgG1, 6/14 with disease vs 11/11 for isotype control, 101.4, p=0.0029, Fishers exact test). There was no statistically significant delay in the onset of the relapse phase of disease for those animals which entered relapse (a).

5   A significant reduction in weight loss (b, p=0.0001, Student's T test), maximum clinical score (c, p=0.0028, Mann-Whitney U test)) and cumulative clinical score (d, p=0.0001, Mann-Whitney U test) were observed during the relapse phase.

ii) Dosing through the relapsing phase: A reduced incidence, delayed onset and reduced

10   severity of the relapse phase was observed (see figs 4, 5 and 6). Figure 5 shows that the dose groups selected to have a similar acute phase profile, showed no significant differences in any of the parameters analysed. Figure 6 shows that Ab#13 mIgG1 dosed through the relapse phase caused a significant reduction in the incidence of the relapse phase of disease (Ab#13 mIgG1, 5/12 with disease vs 12/12 for isotype control, 101.4, p=0.0046, Fishers exact test).

15   There was also a statistically significant delay in the onset of the relapse phase of disease for those animals which entered relapse (a, p=0.0061). A significant reduction in weight loss (b, p<0.0001, Student's T test), maximum clinical score (c, p=0.0011, Mann-Whitney U test)) and cumulative clinical score (d, p=0.0023, Mann-Whitney U test) were also observed during the relapse phase.

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Summary

Ab#13 mIgG1 antibody was dosed in separate experiments over the acute phase of disease (prophylactic dosing) and over the relapse phase (therapeutic dosing). Effects were most pronounced on relapse with a significant reduction in the incidence and severity of the

25   relapse phase for both dosing regimes.

Example 3. Effect of Ab#13 Fab-Di-PEG on the symptoms of EAE

A Fab fragment, termed Ab#13 Fab-Di-PEG was produced essentially as described in

30   International Patent Application PCT/GB2004/002810 (filed on 1st July 2004). The Fab consisted of the rabbit variable regions of antibody 13 from example 1 and mouse IgG1 constant regions. In contrast to other Fab fragments, the heavy chain constant region of this Fab terminates at the interchain cysteine of C_H1. PCR primers were designed based on the murine IgG1 CH1 region and PCR mutagenesis used to insert a stop codon immediately

following the interchain cysteine of C$_H$1. The mouse constant regions are shown in Figure 13 and in SEQ ID Nos 3 (heavy chain) and 4 (light chain). PCR mutagenesis was also used to replace the cysteine at position 80 of the rabbit light chain variable region with alanine.

5   **Murine CH1 (SEQ ID NO:3)**
KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVL
QSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRD<u>C</u>*

Murine Kappa (SEQ ID NO:4)
10   DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQ
DSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRGE<u>C</u>*

The Fab fragments were produced in *E.coli* strain W3110 and purified using standard methods (Humphreys et al., 2002, Protein Expression and Purification, 26, 309-320).

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2x20kDa PEG was attached to the Fab fragment by attaching a linear 20kDa PEG to each of the interchain cysteines (underlined in the sequences above). Reductions and PEGylations were performed in 50mM Tris.HCl 5mM EDTA pH 7.14 with Fab at 20.06mg/ml. The Fab was reduced at room temperature (~24°C) for 30 minutes using 10mM tris(2-

20   carboxyethyl)phosphine (TCEP) (final). The Fab was desalted on a PD-10 column (Pharmacia) and then mixed with 4 fold molar excess of linear 20kDa PEG-maleimide over Fab. The 20 kDa PEG was from Nippon Oils and Fats (NOF). PEGylated Fab was separated from unPEGylated Fab by size exclusion HPLC on analytical Zorbax GF-450 and GF-250 columns in series. These were developed with a 30min isocratic gradient of 0.2M phosphate

25   pH 7.0 + 10% ethanol at 1ml/min and Fab detected using absorbance at 214nm and 280nm.

The MS model, experimental autoimmune encephalomyelitis (EAE), was used essentially as described by Baker et al., 1990. Journal of Neuroimmunology, 28:261-270.
Female ABH mice 8-10 weeks of age (Harlan) were immunised with mouse spinal cord

30   homogenate (SCH, 3.33mg/ml) in complete Freund's adjuvant in two sites, sc, on the flanks (150µl/site) on days 0 and 7.
Four groups were dosed prior to onset of first relapse (during remission). Groups were assigned as follows –

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Group 1 (n=11) anti-mouse IL17 Ab#13 Fab-di-PEG (100mg/kg, s.c, weekly)

Group 2 (n=11) anti-mouse IL17 Ab#13 Fab-di-PEG (30mg/kg, s.c, weekly)

Group 3 (n=9) anti-mouse IL-17 Ab#13 mIgG1 (10mg/kg, sc)

Group 4 (n=9) PBS control

5

Weights and clinical scores were recorded daily and terminal EDTA-Plasma collected.

Clinical score scale

0	Normal
0.25	Tail dragging
0.5	Partial tail paralysis
1	Complete tail paralysis
2	Incomplete hind limb paralysis/loss of righting reflex
3	Complete hind paralysis / incontinence
4	Front limb paralysis / moribund

Statistics

Pair wise comparisons of maximum and cumulative clinical scores and day of onset were performed using Mann-Whitney U test. Cumulative clinical score is defined as the sum of clinical scores throughout the disease course for each animal (area under the curve). Comparisons of disease incidence were performed using Fishers Exact Test. Maximum weight loss was analysed using one-way Anova with Bonferroni post test.

Results

Figure 7a shows the effect of anti-IL17 Ab#13 di Fab-PEG and Ab#13 mIgG1 on clinical disease when dosed from remission (black arrowheads represent dosing days). When dosed prior to first relapse all active doses showed a significant reduction in cumulative and maximum clinical score and incidence.

Figure 8 shows that during the acute phase, prior to antibody treatment, all assigned groups showed no significant differences in disease onset or clinical severity prior to antibody dosing.

Figure 9 shows that anti-mouse IL-17 antibodies dosed prior to relapse onset during remission showed a significant reduction in maximum clinical score (Ab#13 di Fab-PEG 100mg/kg vs. PBS $p < 0.05$ and IL-17 Ab#13 mIgG1 vs. PBS $p < 0.001$),

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There was also a reduction in cumulative score (Ab#13 di Fab-PEG (100mg/kg and 30mg/kg) and Ab#13 mIgG1 vs. PBS p<0.01, p<0.05 and p<0.001 respectively). Furthermore there was a reduction in maximum weight loss (Ab#13 di Fab-PEG 100mg/kg and Ab#13 mIgG1 vs. PBS both p<0.05).

5

Actual incidence of relapse is summarised in table 1, with all actively treated groups having significantly lower incidence than the PBS control group.

	Ab#13 di Fab-PEG 100mg/kg***	Ab#13 di Fab-PEG 30mg/kg*	Ab#13 IgG1 10mg/kg***	PBS
Animals entering relapse	0	2	0	7
Animals not entering relapse	11	9	11	2
Total number of animals	11	11	11	9

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                   *** P=0.005   *p=0.0216   *** P=0.005

Table 1.

Summary

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Anti-mouse IL17 antibodies (Ab#13 di Fab-PEG and Ab#13 mIgG1) were dosed in a dose dependent manner during the remission phase prior to onset of first relapse. Effects were pronounced with a significant reduction for both antibodies in relapse incidence and upon maximum and cumulative disease score in comparison to the PBS control group.

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Example 4. Effect of Ab#13 mIgG1 on the symptoms of Chronic EAE in C57Bl/6 mice.

The chronic EAE model used was essentially as described by Copray et al., 2004. Journal of Neuroimmunology, 148:41-53.

Female C57Bl/6 mice 6-8 weeks of age (Charles River) were immunised with Myelin

25 Oligodendrocyte Protein (MOG 35-55, 0.66mg/ml) in complete Freund's adjuvant (0.4mg/ml Mycobacterium; 4:1 M.tuberculosis: M butyricum) in two sites, s.c, on the flanks (150µl/site) on days 0 and 7. Mice were also administered pertussis toxin (1µg/ml) on days 0,1,7,8; 200µl i.p

Two groups were dosed prophylactically (10mg/kg; s.c; commencing on PSD −1until the end of experiment). One group (n=15) was dosed with Ab#13 mIgG1, (chimeric rabbit V region, Murine constant region IgG-1, as described in Example 1), the other (n=15) with 101.4 (Murine IgG-1 isotype control, 101.4).

5 Two groups were dosed therapeutically (upon 50% incidence) with Ab#13 mIgG1 and control antibody 101.4 (10mg/kg; s.c commencing PSD 16 till end of experiment) Weights and clinical scores were recorded daily and terminal EDTA-Plasma collected.

Clinical score scale

10   0   Normal
     0.25   Tail dragging
     0.5   Partial tail paralysis
     1   Complete tail paralysis
     2   Incomplete hind limb paralysis/loss of righting reflex
15   3   Complete hind paralysis / incontinence
     4   Front limb paralysis / moribund

Statistics

Statistical analysis was performed as described in Example 3.

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Results

Figure 10 shows that prophylactic dosing (a) of Ab#13 mIgG1 elicited significant delay in the onset of disease, cumulative and maximum clinical score. Therapeutic dosing (b)

25 significantly reduced cumulative clinical score. Further analysis showed Ab#13 mIgG1 dosed prophylactically had significant effects in reducing cumulative score (p=0.0006), delay of onset (p=0.0184) and maximum clinical score (p=0.0032, all Mann-Whitney U test) (Figure 11). Ab#13 mIgG1 dosed therapeutically also showed a significant reduction in cumulative score (b, p=0.0229, Mann-Whitney U test; Figure 12).

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Summary

Anti-mouse IL17 antibody Ab#13 mIgG1 was dosed in separate experiments prophylactically and therapeutically. Effects were most pronounced prophylactically with a significant reduction in maximum and cumulative disease score, furthermore incidence of

35 disease was significantly delayed. Therapeutic treatment showed a reduction in cumulative disease score.